THE EFFECTS OF FLOW ON SWIMMING BEHAVIOR OF BRACHIONUS MANJAVACAS (ROTIFERA)

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THE EFFECTS OF FLOW ON SWIMMING BEHAVIOR OF BRACHIONUS MANJAVACAS (ROTIFERA)

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SUMMARY

Rotifers serve as model species and are crucial to the zooplankton communities in terms of feeding and nutrition as well as their overall contribution to aquatic food webs (Wallace et al., 2010). Rotifers experience fluid flow in their natural environments of lakes and streams. Fluid velocity acts as stimulus to rotifers, causing them to adjust their swimming speed and direction. I am interested in how rotifers respond to flow, which is known as rheotaxis (Marcos, 2012). *Brachionus manjavacas* is the rotifer species employed in my experiments. This study simulates fluid flow at rates similar to that rotifers may experience in a riverine ecosystem with unidirectional flow. My intention is to uncover the ways in which the animals respond to flow in these tightly controlled conditions. Rotifers are categorized by age and tested in flow rates ranging from 0.0 to 1.0 mm/sec. Video analysis enables us to quantify swimming velocity and dissect its directionality. The study observes *Brachionus manjavacas* behavior in terms of aging and analyzes behavior (swimming) from an ecological perspective. It was observed that two-day-old rotifers swim the fastest on average, while four-day-old animals show fastest swimming patterns against the flow. The end result is a behavioral profile that can be useful for understanding how rotifers adapt to flow.

CHAPTER 1

INTRODUCTION

Introduction to *Brachionus manjavacas*

Rotifers are animals in the Phylum Rotifera that encompasses the zooplankton community and can account for up to half of zooplankton production due to rapid reproduction (Wallace et al., 2010). Rotifers serve as indicator species and are crucial to the zooplankton communities in terms of feeding and nutrition and their overall contribution to the marine food web (Wallace et al., 2010). Specifically, rotifers serve a large role in the diet of larval fish by providing the necessary nutrition the fish require, identifying the rotifers ecological relationship to the fish (Wallace et al., 2010). Additional resources reveal rotifers are good model organisms because are easy to culture in lab, have relatively short life spans, and play an important role ecologically. *Brachionus manjavacas* is the rotifer species highlighted here; this particular species thrives in saltwater environments and spends its entire life swimming in the plankton. Rotifers also have been good models for studying aging, and this study will focus on the rotifer *Brachionus manjavacas* and how this rotifer modifies its swimming behavior with aging. The end goal is to develop a behavioral profile that can be used for understanding how rotifers modify their swimming behavior in response to flow and how this affects their ecology.

Importance of Research on Aging and Behavior

In the article, "Rotifers as models for the biology of aging", Snell introduces the idea that rotifer aging research could discover new genes and pathways affiliated with human aging, and thus correspond with the overall goal of developing innovative techniques to extend human life (Snell, 2012). According to Snell (2012), rotifer aging is

associated with structural, behavioral, and metabolic changes. Behavioral changes are emphasized in the current study. Physiological and ecological processes can be linked by behavior (Scott, 2004); this is shown by "behavior endpoints" which reveal effects (alterations in biochemical and physiological processes) due to chemical exposure (Oliveira, 2013). By building a primary behavioral profile initially, toxicity tests can be conducted in the future to document changes in animal behavior and further understand the physiological processes at hand and draw ecological conclusions.

Importance of Locomotive Behavior-Swimming

As behavior links physiology with ecology, locomotive behavior links physiological, metabolic and neurological processes with the anatomical state (Oliveira, 2013). Because of the link between locomotive behavior with other behaviors, locomotion will be targeted for investigation.

While reviewing locomotive research, it is important to acknowledge rotifer habitats to understand that swimming is the primary form of rotifer locomotion. To begin, it is known that rotifers can be found in rivers all over the world (Lair, 2006). The animals inhabiting riverine ecosystems encounter flow and it serves as a stimulus (Suss, Cobbs, and Thorp, 2008). Rotifers are capable of swimming due to their cilia and they can mediate the speed of their swimming by the rate at which they beat the locomotory cilia (Gilbert, 1988). They have the potential to swim fast in the current simulated environment while facing the flow or turning against it, swim slowly while allowing for the flow to move them along their path, or remain stationary by attaching to a substrate with their foot in a way that prevents displacement.

Adhesive glands at the tip of the foot enable attachment as well as controlled detachment (Lair, 2006).

As swimming is the primary form of rotifer locomotion, swimming speed and direction will be dissected in this study. Researcher Oliveira (2013) states the effects of chemicals and the physiological state of animals are most commonly analyzed by swimming velocity (Oliveira, 2013). Swimming performance can be a large factor in determining aquatic animals' survival considering its strong influence in food gathering, reproduction, and avoidance of unfavorable conditions.

The Effects of Fluid Velocity as an Environmental Stimulus

Fluid velocity acts as stimulus to rotifers' swimming speed and direction, and rotifer rheotaxis refers to their swimming (behavioral) response to this fluid movement (Marcos, 2012). There are positive and negative ecological consequences to their rheotaxis such as potential increases in food availability, predator encounter, oxygenation of the water, dispersion of resting eggs, and the energy cost of swimming as well as a potential decrease in feeding efficiency (Lair, 2006). Amatzia (2005) indicates the differing consequences can be explained by depth maintenance; swimming allows rotifers to maintain their depth and become positioned within certain layers of their aqueous habitats and thus experience differing circumstances depending upon their layer of encounter. Components of rotifer habitats such as food, predator, and oxygen concentration as well as light intensity, temperature, and water movement are not

consistent throughout the habitat, explaining rotifer positioning within these habitats (Snell, 2012).

Bertani et al.'s survey highlights monogonont rotifers, a group including saltwater rotifer species, and the lack of abundant biogeographical knowledge of the group in terms of ecology and distributional patterns (Bertani, et al., 2011). In comparison to other environments rotifers inhabit, there is less data available on riverine ecology of rotifers (Lair, 2006). Due to the gap in this field of research, the need for this ecologically based study on rotifer rheotaxis is promoted.

This project focuses on investigating rotifer swimming behavior in response to fluid velocity. Specifically, how will rotifers swim at increasing flow rates and how does age affect this response. A survey of *Brachionus manjavacas* populations in tanks with varying levels of turbulence found high turbulence tanks allowed for the fastest growth of the population (Sluss, Cobbs, and Thorp, 2008). This leads us to hypothesize the rotifers will have strong swimming responses under strong currents. We also predict their responses will have significant ecological impacts considering similar species copepods and dinoflagellates rely on rheotaxis for attacking prey, depth retention, predator escape, and orientation (Marcos, 2012). We expect rotifers will show the strongest swimming at four days old in accordance with the high swimming speed values observed in the absence of flow (Snell, 2012). Specifically, we anticipate *Brachionus manjavacas* four day olds to possibly reach a top swimming speed around 1.8 mm/sec because this was the highest speed reached by the rotifer species *Keratella* due to avoidance behavior (Gilbert, 1988). We do not expect *Brachionus manjavacas* to surpass this value. *Keratella* swimming speeds ranged around 0.5 mm/sec on average, and this gives us reason to

surmise values around 0.5 mm/sec may be reached by *Brachionus manjavacas* species in this study (Gilbert, 1988). Washout or loss of control due to the current in *Brachionus manjavacas* testing is another factor for consideration. Marine organisms *Daphnia* and *Eucyclops* experience washout at 2.50 cm/sec and 7.75 cm/sec flow rates respectively, and this leads us to suspect Brachionus manjavacas may experience washout at similar stimulus strengths (Richardson, 1992). In conclusion, we hope to draw ecological conclusions from these observations and implement them in future aging research.

CHAPTER 2

METHODS

Brachionus Manjavacas Culture

Rotifer species *Brachionus manjavacas* were hatched from resting eggs and fed *Tetraselmis suecicia*. They were separated in 24 well plates based on age class with five animals in each well. Each well contained 1 mL of liquid with approximately 5 um of chemical 5-fluoro-20-deoxyuridine (FDU for reproduction prevention) and *Tetraselmis suecicia* concentrated at 2 X 10^6, along with Artificial Salt Water (ASW) at 15 ppt. The rotifers were kept at a temperature of 22^{o}C in an environmental growth chamber where they aged. The day the animals reached the desired age for experimentation, those animals were isolated into a dish only containing artificial salt water (salinity of 15 ppt) to wash the animals.

Fluid Velocity Simulation

The animals underwent testing one at a time. The first animal was placed into a polymer channel,10.0 cm in length/500 uM in diameter, designed by students apart of a chemical engineering lab at the Georgia Institute of Technology. The polymer channel was attached to tubing (0.580 mm in inner diameter) that connected to the syringe component of a syringe pump. The 10.0 mL syringe was 1.50 cm in diameter and the syringe pump controlled the rate of flow ASW traveled through the tubing into the channel inhabiting the rotifer. The individual rotifer was placed at the front end of the channel and the entire component was placed under a light microscope set at 0.9x magnification. The microscope included an attached camera, model P1A642, that connected to the computer using Pixelink software and filmed the rotifers under a frame

rate of fifty frames/sec. Ten animals were recorded for thirty-second time intervals under each flow rate (no flow, 0.0108, 0.0223, 0.303,0.0508 mm/sec), with a new group of ten animals recorded as the flow rates were increased. The animals were tested at the ages of 2, 4, 8, and 12 days old.

The recorded videos (.avi format output) were converted into sequences of images through Virtual Dub and the images were edited through an Image J program. Meijering et al.'s (2012) guide served as the source in performing particle tracking techniques, which in this case the rotifers served as the tracked particles. The images produced through Image J required a cleared background where components of the channel and other background objects captured by the camera could not be detected. Through this process the animal would be clearly highlighted and would appear as a white object on a black background. Image J plugins Image Stacker Merger Plus and Mtrack2 aided in the background clearing as well as rotifer movement tracking by providing the rotifers' position through coordinate values (x,y coordinates) in each image frame. The coordinates were stored in a .txt file and plotted as a path.

The values were converted in Excel files to determine the speed and change in direction during each time period. Original values were measured in pixels/frame and we converted this to mm/sec and bodylength/sec units. This was completed by determining the amount of pixels in one millimeter physically measured on a ruler placed under the microscope at the same magnification of 0.9x. By detecting the amount of pixels in this one frame of reference we obtained mm/sec values that were converted to bodylengths/sec in accordance to Campillo et al.'s (2005) measurement. Campillo et al. identifies the lorica length of *Brachionus manjavacas* as 360.7 um and using this value

allowed for value transformation from millimeters to body lengths. Speed was calculated in the negative and positive direction, indicating how quick the animals' movements were when swimming against the flow (positive) and when swimming with the flow (negative). A difference in velocity was calculated to distinguish the direction in which the animals swam fastest. Converting these swimming values to units of body lengths/second allowed for swimming speed comparison among animals relative to rotifers.

CHAPTER 3

RESULTS

Image J software and plugin use transferred videos into image sequences to allow for the videos to be completely broken down and for rotifer movement to be tracked through each frame of movement. Coordinate values (x and y) were provided for each video and allowed for velocity calculation through Microsoft Excel. Values were recorded for overall swimming velocity of *Brachionus manjavacas* under each flow rate for each age class, as shown through Figure 1 below. Essentially, Figure 1 allows for interpretation as to what highest swimming speeds were reached and from what age and flow rate group these speeds derived from.

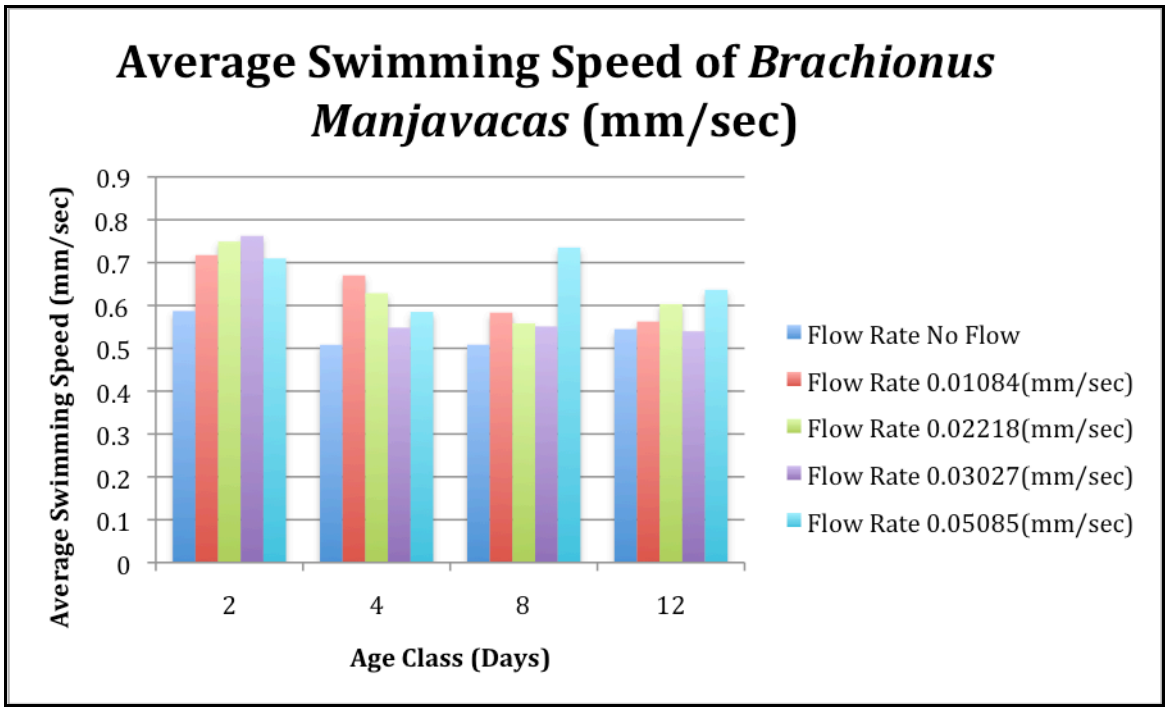

Figure 1: The mean swimming speeds of two, four, eight, and twelve day old animals under flow rates ranging from no flow (0.0 mm/sec) to high flow (0.0508 mm/sec).

Figure 1 identifies two-day-olds as the youngest age class and twelve-day-olds as the oldest, with two-day-olds holding the fastest swimming averages overall. Two-day-olds did not show drastically high swimming speeds when the stimulus was not available (no flow). The eight and twelve-day-olds showed an increase in speed under 0.0508 (mm/sec) flow rate. To continue swimming speed analysis, calculations were performed to show swimming speed in terms of direction; thus depicting whether rotifers swam faster in the direction of the current or when traveling against the current. This is understood as the mean difference in the rotifers' swimming patterns and was calculated as the average speed of the animals in the "positive" direction, or with the current, minus the average speed in the opposing direction when the animals swam against the current.

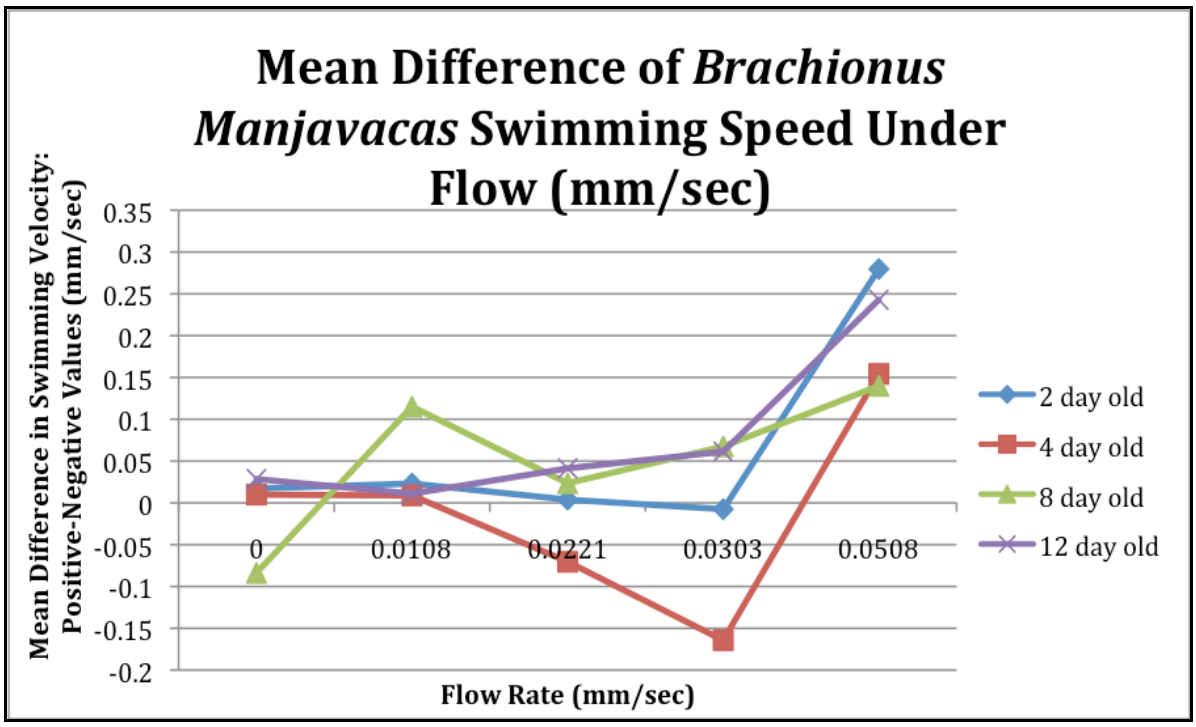

Figure 2: Mean difference in swimming velocity of *Brachionus manjavacas* from each age class under flow rates ranging from 0.0 to 0.0508 (mm/sec).

The four-day-old rotifers swam the fastest in the direction facing the stimulus in comparison to the other animals (Figure 2). This is illustrated by the steep decrease in the

change to mean swimming velocity at 0.0303 (mm/sec) that is lower in comparison to the other age classes. Additionally, the sharp increase in change of swimming velocity at the 0.0508 (mm/sec) flow rate was the point in the simulation when the rotifers experienced washout due to the stimulus. The twelve-day-old rotifers held higher positive average swimming velocity difference than the other age classes overall, confirming that they swam fastest in the direction of the current as opposed to when they were facing it.

Due to the results from the average swimming velocities and mean differences amongst the rotifers, we segregated the four and twelve-day animals at the lowest and highest flow rate to pin point distinctions in path patterns. Because two day olds swam fastest overall (Figure 1), we obtained paths from varying flow rates as well to observe behavioral changes as stimulus strength augmented.

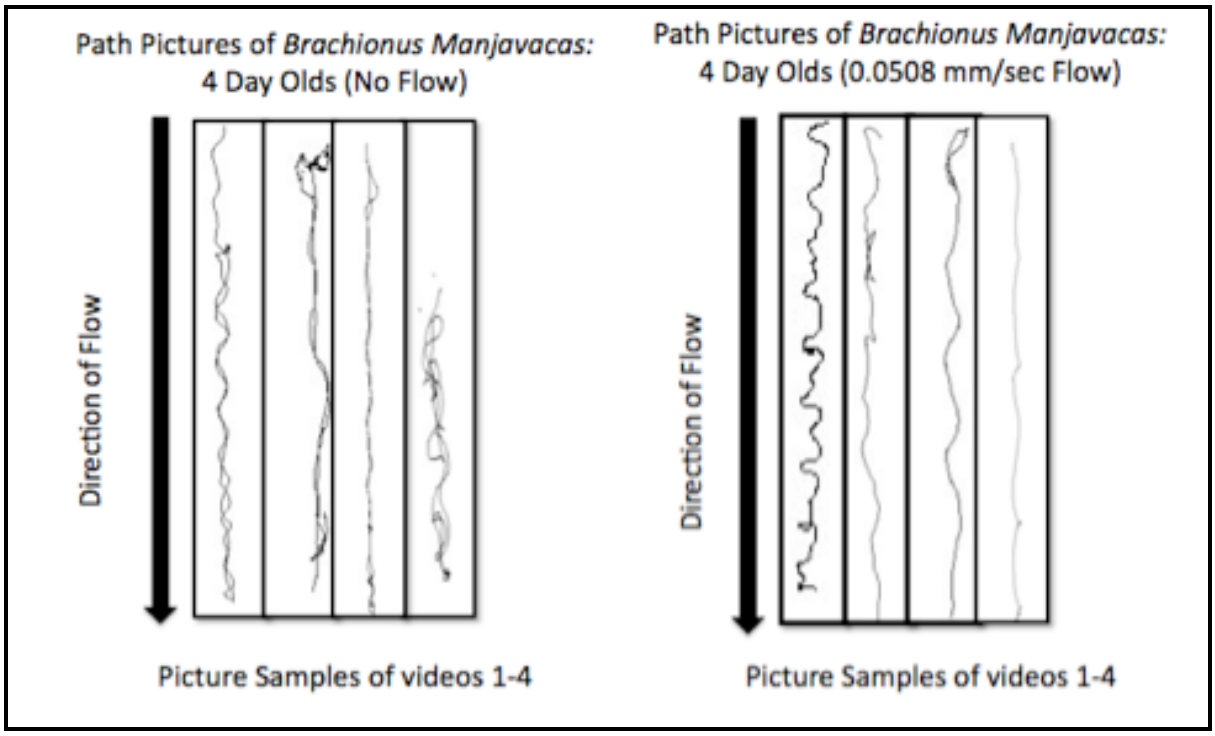

Figure 3: Path picture comparison of four day old animals swimming without influence from fluid stimulus and four day old animals swimming under the highest set flow rate of 0.0508 (mm/sec).

The difference in path patterns is clearly recognized as the flow rate increases in the four-day-old animals (Figure 3), which were chosen for comparison here due to high velocity when swimming against the current. Four-day-old animal results were compared to results from the oldest age class, twelve-day-old, where swimming velocity values against the current were much less meaningful. These are identified through path pictures in Figure 4 for the twelve-day-old animals.

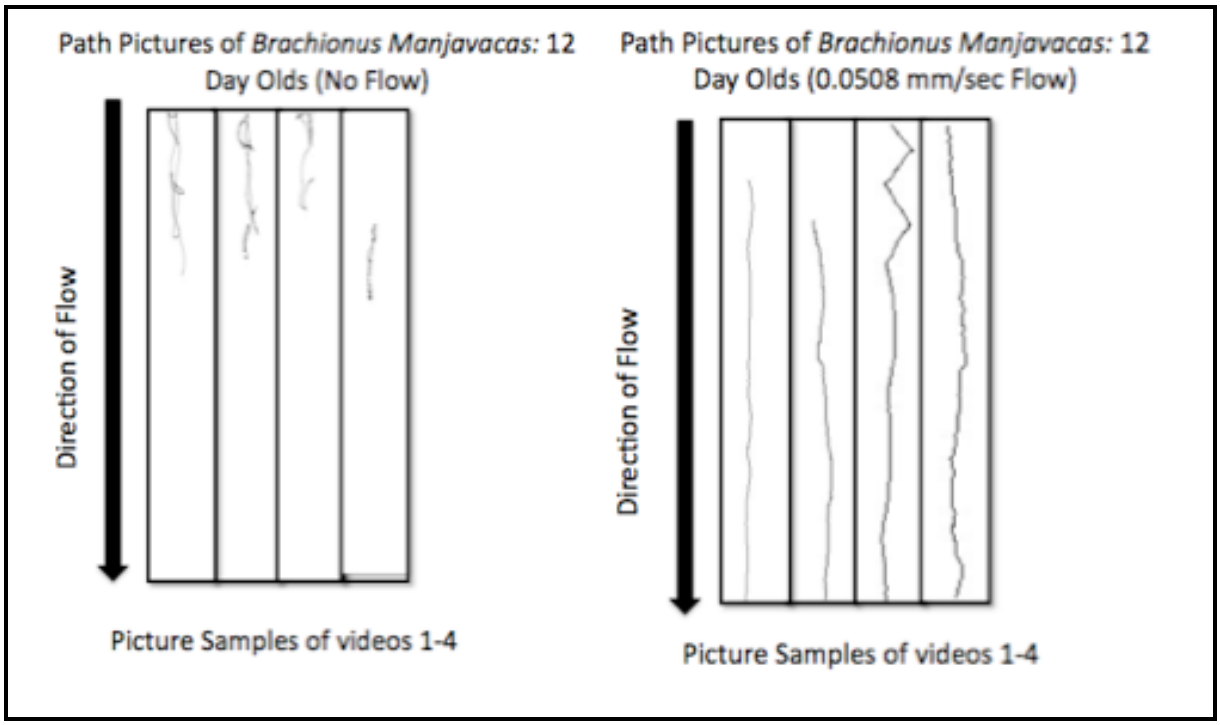

Figure 4: Path picture comparison of twelve day old animals swimming without influence from fluid stimulus and twelve day old animals swimming under the highest set flow rate of 0.0508 (mm/sec).

Figure 4 exposes that the path patterns vary significantly as the stimulus strength increases. The animals initially show swimming patterns in zigzags or loops and change to nearly straight lines under high flow. Figures 3 and 4 depict the difference in rotifer rheotaxis amongst the age groups.

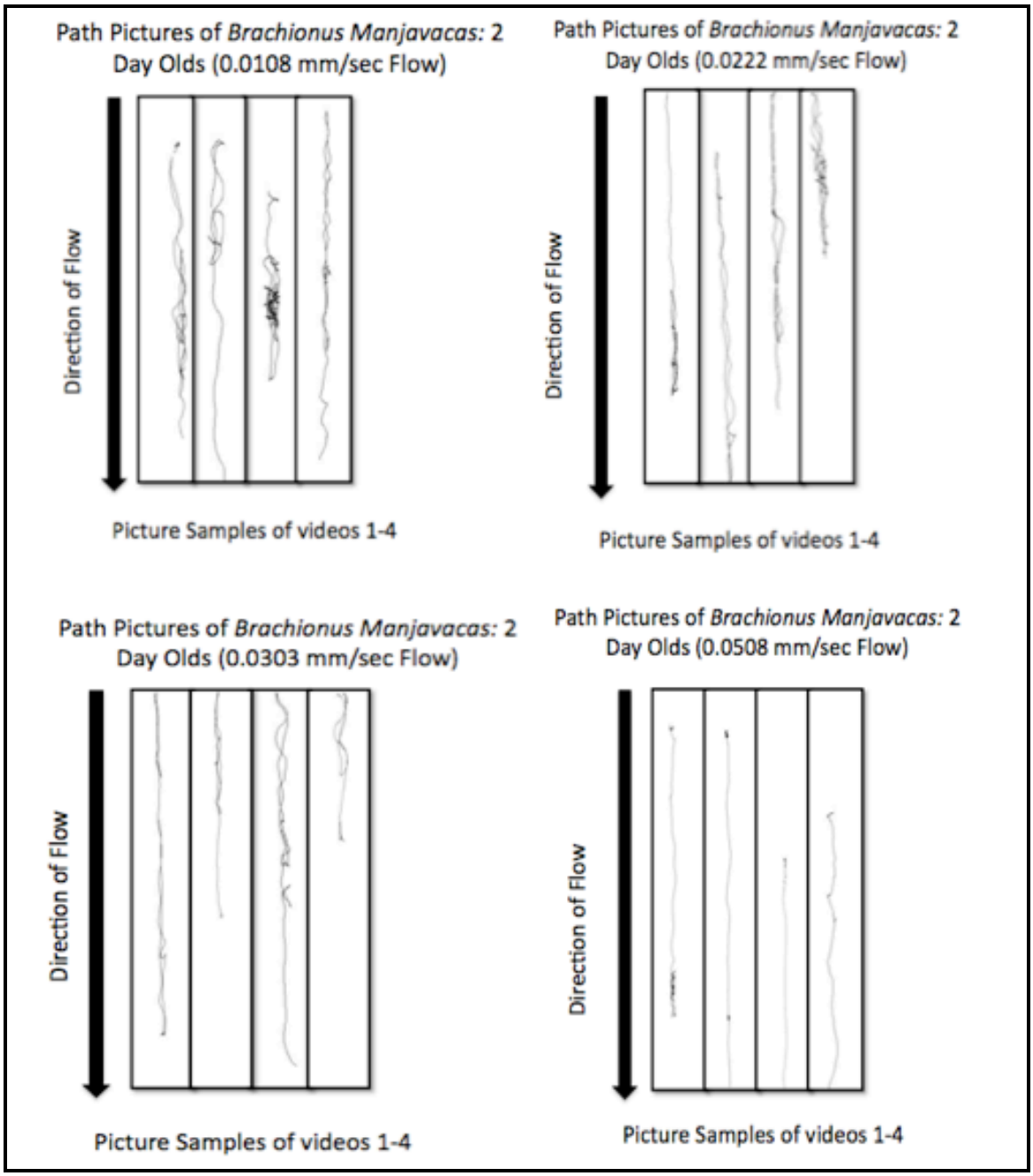

Figure 5: Path pictures of two-day-old animals under each flow rate excluding no flow where average swimming speeds were significantly lower than speeds recorded under other flow rates.

Distinct behavioral differences within the two-day-old age class occurred under different flow regimes (Figure 5). The paths show rapid movement in the lower flow rates by the intense zigzag patterns; the more narrow pathways with less back and forth movement

suggest rapid movement was not as attainable and caused the animals to follow a straighter route.

CHAPTER 4

DISCUSSION

Figures

This study is a video analysis of *Brachionus manjavacus* from differing age classes exposed to varying strengths of a fluid velocity gradient. The fluid velocity acts as a stimulus similar to that present in rotifers' natural habitats of rivers and lakes. Through video analysis, rotifer rheotaxis was examined and from these observations ecological conclusions can be drawn; such as those pertaining to the animals' attempt to maintain depth and position within their environment to pursue food, mates, etc. (Amatzia, 2005).

Average Swimming Speeds

Through the study we show the youngest animals making up the two day old age class swim fastest on average with swimming speeds peaking 0.75 (mm/sec). Only under 0.0508(mm/sec) flow was it apparent that the eight-day-old animals swam faster than the two day olds (Figure 1). The higher swimming velocities of the older animals overall from this flow rate may be explained by animal washout at the high rate as opposed to the option that the animals were displaying stronger swimming performance. We suggest at this high flow rate the oldest animals (eight and twelve day olds) are susceptible to washout due to fatigue and lack of strength to swim against the current. Ecologically, we propose this serves as a disadvantage to aged animals in that they cannot control their position in their aqueous habitat and thus may not be able to escape predators, obtain food, etc. as efficiently as the two and four day old rotifers. Exact washout values were not in agreement with our hypothesis considering that under 0.0508 mm/sec flow rate the

rotifers experienced washout and this fell far out of our estimated range of 2.50 and 7.75 cm/sec flow rate (Richardson,1992).

The two-day-old animals reached their peak swimming speed under 0.0303 (mm/sec), and we suggest this is due to a rheotaxis promoting them to swim faster when a stimulus is present in their surroundings. The four-day-old animals swam faster on average when the current was present than under no flow (Figure 1). The overall swimming average of this age class does not dramatically change as flow increases, but the significance of their behavior in terms of swimming direction as the stimulus becomes stronger is emphasized by the mean difference evaluation. The fact that the four-day-old animals do not swim faster than those making up the other age classes contradicts our initial prediction, considering we proposed the four day olds would perform strongest overall.

The eight-day-old animals did not show a steep behavioral alteration as flow rate increased until the highest rate was reached, where the animals then showed increased swimming speed which may again reflect washout effects. This data was consistent with the twelve-day-old animals as well.

Mean Difference

The Mean Difference graph depicts the rotifers' rheotaxis in terms of swimming speed and direction (Figure 2). The graph can be interpreted as the positive velocity values indicating the portion of swimming performed in the direction of the flow whereas the negative values indicate when the animals faced the flow to swim against their introduced fluid velocity gradient. These graphs proved most helpful in analyzing rheotaxis in that it is clearly distinguishable as to which age classes were capable of

fighting the gradient and performed accordingly and which age classes may have been incapable and thus held lower velocity averages overall or were instead completely swept away.

The two-day-old animals show a mild peak in the negative direction under 0.0303(mm/sec) flow, suggesting that as the flow increased they showed a greater attempt to swim faster in the direction of the stimulus. This trend was followed to a greater extreme in the four-day-old age class; the rotifers at this age measured a mean difference of -0.164(mm/sec) which was the greatest negative value throughout the age classes (Figure 2). We interpret this value as an indicator that the rotifers were intentionally facing the stimulus and increasing their swimming speed (?). We suggest these younger animals compliment our hypothesis in that they are found capable of swimming at high speeds against the stimulus due to their health at this age (?). This leads us to conclude that in their natural environments, this species of rotifers may be able to swim against the current and maintain their depth (Amatzia, 2005). This form of rheotaxis may allow for the rotifers to sustain themselves within favorable layers of their aquatic habitat and exploit resource-rich patches (Amatzia, 2005). Similarly, we predict that in their natural environments, the rotifers exhibiting swimming weakness may form less dense patches due to fatigue and lack of strength to swim fast and maintain depth and position (Amatzia, 2005). In effect, four-day-old animals may hold the advantage of receiving the positive consequences from depth retention such as mate recognition, food availability, and predatory escape due to rheotaxis (Lair, 2006). This age class also supports the concept of measuring how greatly an organism may benefit from its motility due to rheotaxis (Marcos, 2012). The four day old animals showed swimming preference

in the direction of the fluid velocity gradient closer to 0.0508 (mm/sec) flow, and this implies the gradient may have become too strong for the rotifers to completely control their positioning and thus were susceptible to being swept down the channel. Because of the negative value we also concur the animals may have spent more time facing the direction of the flow in order for the values to measure out like they did.

The results from the four day olds promote the basis for this study; the analysis of swimming velocity as a form of locomotion depicts animal behavior and may further reveal physiological and ecological links (Scott, 2004). Through this understanding, these results could be beneficial in future testing involving swimming studies and changes in rotifer behavior.

Animals falling in the older age classes of eight and twelve day olds experienced similar responses in that they held positive averages under each flow rate. The eight day olds show a peak at 0.0108 (mm/sec) but this value may be insignificant in that the strength of this current should not have dramatically affected behavior. Under the proceeding flow rates, the mean difference values remained positive revealing the animals swam in the direction of the flow faster than when they faced the flow. The eight and twelve day old animals both exhibited the highest positive difference under 0.0302 (mm/sec) as well with values of 0.067 (mm/sec) and 0.061 (mm/sec) respectively. Twelve day olds resulted in a higher difference under 0.0508 (mm/sec) flow, indicating they may have been less capable of fighting washout by the strong current than the eight day olds. From these age classes we suggest higher swimming velocities in the direction of the stimulus will lead to different ecological consequences than those experienced by the younger animals. We propose the older animals may fatigue quicker under strong

flow (?); and they may have less control of position and depth maintenance. This leads us to surmise older rotifers may be subject to negative ecological consequences (Amatzia, 2005). Also, these results supported our hypothesis in that because the older animals cease reproduction, they may not be in their strongest phase of life and cannot perform as well as younger animals (). These results will help for future predictions regarding rotifer behavior under toxicity stress in that we will be able to expect for younger animal performance to surpass that of the older animals.

Paths

Four day old path pictures were chosen for analysis because of the steep peak in the negative values under mean difference calculations. Not suprisingly, their path pictures resulted in patterns we expected from these high velocities against the stimulus. Under 0.0 mm/sec flow, the rotifers show drastic movements from the top of the channel (closest to the tubing) to the bottom of the channel, and this can be seen by the double zigzag lines that overlap. The animals do not follow a straight or narrow path. As flow is increased to 0.0508 mm/sec flow, an overlap of two or more zigzag lines is no longer apparent and only one line is followed through the channel. Although one picture indicates a dramatic zigzag pattern, video samples 2-4 reveal a straighter pathway. We suggest this difference in path pattern coordinates with the animals' fast swimming patterns against the flow and the slight zigzag may be due to the high current having somewhat of a control on their swimming direction. For the straighter paths we suggest this as the rotifers' attempts in orienting themselves within the current.

In comparison, twelve day olds show almost perfectly straight paths under 0.0508 mm/sec rate. We suggest that instead of this acting as a method of orientation, that

perhaps the animals had zero control of their positioning under the stimulus and were pushed straight through the channel. This corresponds with the high swimming values in the positive direction as indicated by the mean difference graph (figure 2), in which we suggested the high values were products of washout. In regards to twelve day olds under 0.0 mm/sec flow, it is observed the animals do not show movement from the top to the bottom of the segment of the channel in which they were filmed as drastically as the four day olds. Again, in accordance with the swimming speed and difference graphs, we concur these older animals are weaker swimmers and consistently reveal this through the altering flow rates. We also believe the comparison between the animals we determined to be the weakest and strongest in terms of swimming has led us to distinguish the rheotaxis among the age groups.

Two-day-old path pictures were also chosen for analysis because of their fast swimming speeds overall. Analysis began at 0.0108 mm/sec flow because this is the flow rate in which speed seemed to peak over the other age classes. Under this relatively low flow, path patterns follow our expectations and mimic that of the four day olds under low flow and portray overlapping lines indicating their movement from the top to the bottom of the channel. The middle flow rates portray rapid movement at the front end of the channel where flow was first exposed to the animals, and we believe this coordinates with relatively high values in the negative direction, although not as drastic as the four day olds. As the paths straighten out at the end, we believe this is when the animals began to change position and instead orient themselves with the flow, and this agrees with the less dramatic peak in the negatives considering these positive swimming values would have cancelled some of the negative speed values from the initial point of exposure.

Under 0.0508 mm/sec flow a straight narrow path is witnessed with less zigzag patterns than the four-day-olds, indicating perhaps weaker rheotaxis response to the high flow than the four day olds.

REFERENCES

Amatzia Genin, Jules S. Jaffe, Ruth Reef, Claudio Richter, and Peter J. S. Franks (2005). Swimming Against the Flow: A Mechanism of Zooplankton Aggregation. Science, 308 (5723), 860-862.

Bertani, I., Rossetti, G., & Segers, H. (2011). Biodiversity down by the flow: New records of monogonont rotifers for Italy found in the Po river. *Journal Of Limnology*, *70*(2), 321-328. doi:10.3274/JL11-70-2-N3

Campillo, S., Garcia-Roger, E., Martinez-Torres, D., & Serra, M. (2005). Morphological stasis of two species belonging to the L-morphotype in the Brachionus plicatilis species complex. *Hydrobiologia*, *546,* 181-187.

Gilbert, J., & Kirk, K. (1988). Escape Response of the Rotifer Keratella: Description, Stimulation, Fluid Dynamics and Ecological Significance. *Limnology and Oceanography*, *33* (6), 1440-1450.

Lair, N. (2006). A review of regulation mechanisms of metazoan plankton in riverine ecosystems: aquatic habitat versus biota. River Res. Applic., 22: 567–593. doi: 10.1002/rra.923

Marcos, Fu, H., Powers, T., & Stocker, R. (2012). Bacterial rheotaxis. Proceedings Of The National Academy Of Sciences Of The United States Of America, 109(13), 4780-4785.

Meijering, E., Dzyubachyk, O., & Smal, I. (2012). Methods for cell and particle tracking. imaging and spectroscopic analysis of living cells. *Methods in Enzymology*, *504*, 183-200.

Mohammed, Ryan Simon; van Oosterhout, Cock; Schelkle, Bettina; Cable, Joanne; McMullan, Mark. (2012). Upstream guppies (Poecilia reticulata, Peters, 1859) go against the flow. *Biota Neotropica*, 68-72.

Oliveira, C., Almeida, J., Guilhermino, L., Soares, A., & Gravato, C. (2013). Swimming velocity, avoidance behavior and biomarkers in Palaemon serratus exposed to fenitrothion. Chemosphere, 90(3), 936-944. doi:10.1016/j.chemosphere.2012.06.036

Richardson, W. (1992) Microcrustacea in flowing water: experimental analysis of washout times and a field test. *Freshwater Biology*, *28,* 217-230.

Scott, G. R., & Sloman, K. A. (2004). The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquatic Toxicology, 68(4), 369-392.

Sluss, T. D., Cobbs, G. A., & Thorp, J. H. (2008). Impact of turbulence on riverine zooplankton: a mesocosm experiment. Freshwater Biology, 53(10), 1999-2010. doi:10.1111/j.1365-2427.2008.02023.x

Snell, TW, A. Fields and RK Johnston (2012). Antioxidants can extend lifespan of Brachionus Manjavacas, but only in a few combinations. Biogerontology Epub 2012 Jan 24.

Wallace RL, & TW Snell, (2010). Chapter 8: Rotifera. Ecology And Classification Of North American Freshwater Invertebrates, 173-235. doi:10.1016/B978-0-12-374855-3.00008-X